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Moelleriella pumatensis, a new entomogenous species from Vietnam

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Abstract — *Moelleriella pumatensis*, a fungal pathogen infecting scale insect nymphs (*Hemiptera*), is described and illustrated as a new species from Pu Mat National Park in Vietnam. This species is unique in producing a golden yellow spore mass surrounding the stroma. In surveys throughout the year in Vietnam, only the anamorphic state has been found in the natural forest. Morphological characters and phylogenetic analysis of translation elongation factor 1-α (*tef1*) reveals this species as an anamorph of *Moelleriella.*

Key words — morphology, phylogenetics, taxonomy

Introduction

The genus *Moelleriella* Bres. (*Ascomycota, Hypocreales, Clavicipitaceae*), a fungus pathogenic to scale insects and white flies, was recently segregated from the genus *Hypocrella* Sacc. together with *Samuelsia* P. Chaverri & K.T. Hodge (Chaverri et al. 2008). *Moelleriella* was described based on molecular data and morphology: its ascospores disarticulate inside the ascus, whereas *Hypocrella* and *Samuelsia* ascospores do not. The anamorphic state of *Moelleriella* is *Aschersonia*-like, i.e., similar to *Aschersonia* sensu stricto (teleomorph *Hypocrella* sensu lato; Chaverri et al. 2008). *Aschersonia* sensu lato species are differentiated mostly on the shape and color of the stromata that cover the hosts, pycnidium-like conidiomata, phialides, and presence or absence of paraphyses. These characters have been useful in distinguishing between subgenera of *Aschersonia* (Petch 1921, 1925, Mains 1959, Chaverri et al. 2008). All species of *Hypocrella*, *Moelleriella,* and *Samuelsia* are pathogenic to scale insects (*Coccidae*) or white flies (*Aleyrodidae*) that feed on living leaves and branches of monocotyledonous and dicotyledonous plants.

Exploration of entomopathogenic fungi in Vietnam was initiated under the program of biodiversity studies in Southeast Asia, a collaborative project between the Faculty of Agriculture, Forestry and Fisheries, Vinh University, Vietnam, and the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. One study site, Pu Mat National Park, is located in the southwest of Nghe An Province, an area known to have a high diversity of plants and mammal species. During the surveys in October 2009, an interesting *Aschersonia*-like specimen that produced a yellow mass of conidia covering the stroma was collected from the underside of a leaf of a dicotyledonous plant. The objectives of this study were to 1) evaluate the taxonomic position of the isolates derived from the anamorph state, using the sequences of translation elongation factor 1-α (*tef1*), and 2) describe this specimen as a new species from Vietnam.

Materials & methods

Collection and Isolation

Surveys and collections were made during the rainy season in the Khe Moi trail in Pu Mat National Park (18°46′–19°12′N 104°24′–104°56′E). Material was examined and isolated into pure culture from the anamorphic state following Mongkolsamrit et al (2009). Free-hand longitudinal sections of tissues and conidia were mounted in cotton blue in lactophenol (Heritage et al. 1996) and measured using a light microscope. The color of fresh specimens and cultures were compared with the colors from standard code from Kornerup & Wanscher (1962). Specimens were identified using specialized literature (Petch 1921, Mains 1959, Chaverri et al. 2008). A voucher specimen and culture were deposited in BIOTEC Bangkok Herbarium (BBH) and BIOTEC Culture Collection, Thailand.

DNA extraction, amplification and sequencing

Small pieces of tissue (2–3 mm) of each sample were taken from potato-dextrose-agar (PDA) plates and placed in 50 ml of potato-dextrose-broth (PDB) in a 250 ml flask. Flasks were incubated in a shaker at 200 rpm for 15–20 d at 20°C in dark. The mycelial mass was harvested by filtration (Whatman No.1), washed with sterile distilled water, lyophilized for 1 d, and crushed in liquid nitrogen using a mortar and pestle. Total DNA of each sample was extracted using Cetyltrimethyl-ammonium bromide (CTAB) following the procedure described in Mackill & Bonman (1995). PCR amplification was done in 50 µl volume consisting of 1× PCR buffer, 200 µM of each of the four dNTPs, 2.5 mM $MgCl_2$, 1 U Taq DNA polymerase (Promega, Madison, Wisconsin) and 0.5 µM of each primer. The primers for *tef1* were 983f (Carbone & Kohn 1999) and 2218r (Rehner 2001). Amplifications were performed using a MJ Research DNA Engine ALD1244 thermal cycler following the procedure described in Sung et al. (2001). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. Purified PCR products were sent to Macrogen Inc. Korea for sequencing.

Table 1. List of fungi used in this study.

Species	Voucher/Isolate	GenBank
Balansia henningsiana	GAM 16112	AY489610
Epichloe elymi	C. Schardl 760	AY986951
Hypocrella disciformis	M.L.202i = ARSEF 7695	AY986939
H. disciformis	P.C.655 = CUP 067861	EU392643
H. disciformis	P.C.676 = CUP 067840	EU392645
H. viridans	I89-490 = IMI 346739	EU392649
H. viridans	P.C.632 = CUP 067849	EU392650
Moelleriella mollii	I93-901a = ARSEF 7660	EU392667
M. mollii	I93-901c = ARSEF 7667	EU392668
M. ochracea	IE1308 = P.C.726	EU392669
M. ochracea	P.C.535 = CUP 067777	AY986926
M. ochracea	P.C.626 = CUP 067778	EU392670
M. ochracea	P.C.648 = CUP 067779	EU392671
M. pumatensis	BBH 29281 = BCC 41004	HQ722026*
M. pumatensis	BBH 29281 = BCC 41006	HQ722027*
Samuelsia chalalensis	P.C.560 = CUP 067856	EU392691
S. geonomis	P.C.614 = CUP 067857	EU392692
S. rufobrunnea	P.C.613 = CUP 067858	AY986944

*sequences generated in this study.

Phylogenetic analysis

Sequences were proofed manually, assembled using BioEdit v. 6.0.7 (Hall 2004), and submitted to Genbank (Table 1). Sequences were aligned using ClustalW incorporated in BioEdit and alignments were refined manually by direct examination. Maximum parsimony analysis was performed using random addition sequence (10 replications) and gaps were treated as missing data. Bootstrap analysis was performed using maximum parsimony criterion in 1000 replication samples.

Results

Molecular analysis

Amplification and sequencing of *tef1* were successful for 16 strains comprising two unidentified *Aschersonia*-like specimens. Fourteen selected closely related taxa were obtained from GenBank for this study. Sequences of *Epichloe elymi* and *Balansia henningsiana* were used as outgroup taxa. Of the 899-character alignment in the *tef1* data set, 188 characters were parsimony informative. Maximum parsimony analyses of this data set yielded one parsimonious tree (tree length 436; CI = 0.651, RI = 0.795, RC = 0.518, HI = 0.349) as shown in Fig 1. The phylogenic tree suggests that the two anamorphic isolates with yellow conidial masses (BCC41004 and BCC41006) belong to the genus *Moelleriella* with a strong bootstrap support of 99%.

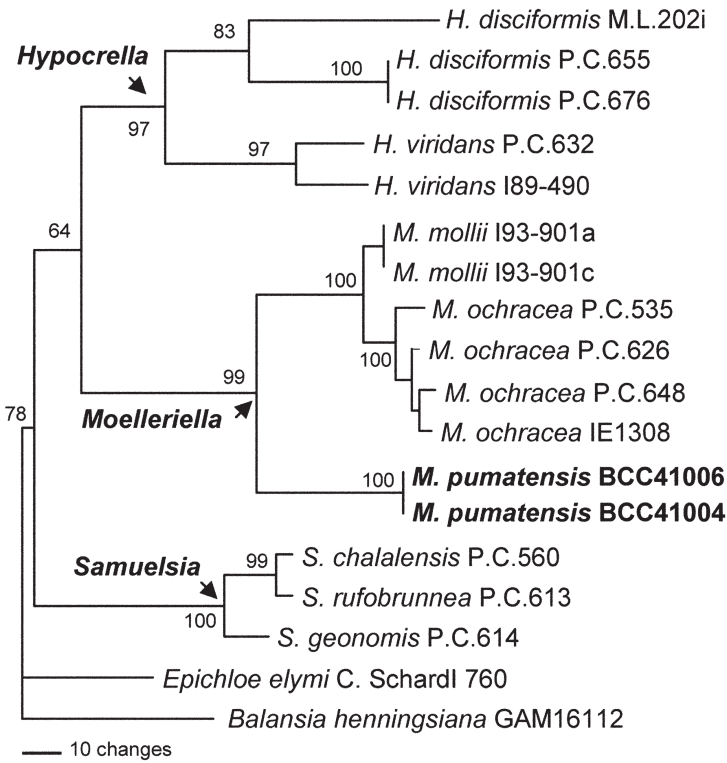

Fig. 1. Phylogenetic relationship of *Moelleriella pumatensis* and related species based on maximum parsimony analysis of the TEF1 gene. Numbers above each branch represent bootstrap support from 1000 replicates.

Taxonomy

Moelleriella pumatensis T.T. Nguyen & N.L. Tran, sp. nov. Fig. 2

MycoBank MB 561074

Stromata discoidea vel pulvinata, ad 2.5 mm diam, 1.5 mm alta, sursum rotundata, aureus, Conidiomata pycnidialia, immerse, disperses, 250–280 µm alta, 90–100 µm diam. Phialides cylindricae, ad 25 µm longae. Paraphyses pycnidiales praesentes, filiformes, flexuosae, ad 180 µm longae, 1.5 µm latae. Conidia fusoidea, utrinque acutata 12–15 × 2–2.5 µm.

Type — Vietnam: Nghe An Province, Khe Moi trail, Pu Mat National Park, on scale insect nymph (*Hemiptera*) on the underside of dicotyledonous leaf, 18 Oct. 2009, J.J. Luangsa-ard, S. Mongkolsamrit, T.T. Nguyen, R. Ridkaew & N.L. Tran (BBH 29281, **holotype**; ex-type culture BCC41006).

Etymology — referring to Pu Mat National Park, the collection location.

Anamorph: *Aschersonia*-like

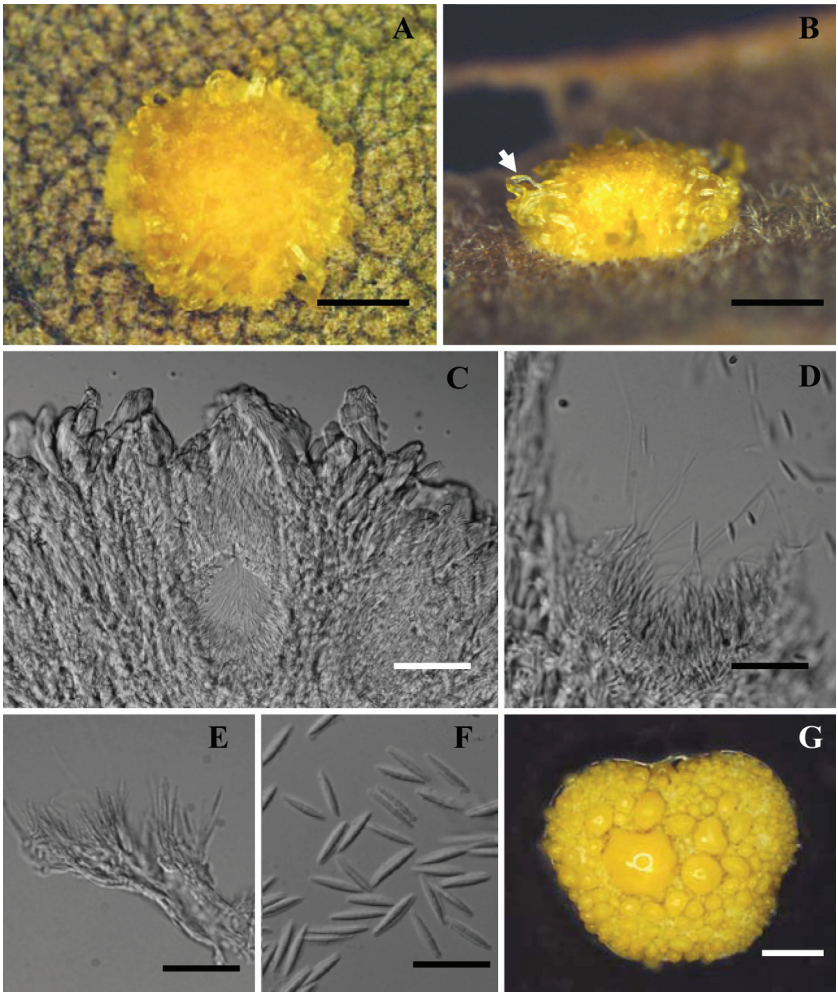

Fig. 2. *Moelleriella pumatensis* A, B, stroma on host showing conidia mass (white arrow); C, pycnidium and sporulating structure; D, E, conidiogenous cells and paraphyses; F, conidia; G, colony on PDA at 20°C after 4 wk (sporulation present). Scale bars: A, B, G = 1 mm; C = 100 µm; D, E, F = 20 µm.

Stromata discoid to pulvinate up to 2.5 mm diam and 1 mm high, upper surface round, golden yellow (Kornerup & Wanscher 1962: 5B7). Conidiomata pycnidial, embedded, scattered in stroma, ovoid, 250–280 µm high, 90–100 µm diam. Conidiogenous cells phialidic, cylindrical, up to 25 µm long. Pycnidial paraphyses present, linear, filiform, flexuous, up to 180 µm long, 1.5 µm wide. Conidia narrowly fusiform, with acute ends, 12–15 × 2–2.5 µm.

Cultural characteristics: Conidia germinating within 24 h on PDA. Colonies on PDA slow-growing, attaining 5 mm diam in 4 wk. Optimal temperature 20–25°C, with no growth at <5°C and >35°C. Stromatic colonies yellow, forming moderately compact stromata. Conidial masses golden yellow (Kornerup & Wanscher 1962: 5B7), appearing as abundant slimy masses from immersed pycnidia scattered over the surface.

Comments: A teleomorphic state of this species was not found in the field although several attempts to find it were made throughout the year. However, the teleomorph name is used for this new species because both phylogenetic analysis and morphology support the placement of this species in *Moelleriella*. In this case, as well as for *Moelleriella madidiensis* from Bolivia also with only an anamorphic state, the teleomorphic name is used (Chaverri et al. 2008). The anamorphic state of *M. pumatensis* is similar to *Aschersonia aleyrodis* (teleomorph: *M. libera*) reported from Brazil, Florida, and Venezuela by Petch (1921) based on pulvinate stroma, yellow mass of extruded spores, and fusiform conidia. The conidia and paraphyses of *M. pumatensis* are somewhat longer; the conidia are 12–15 × 2–2.5 µm and paraphyses up to 180 µm long. In contrast, *A. aleyrodis* has conidia 8–14 × 1.5–2 µm, paraphyses 50–110 µm long. In addition, *M. pumatensis* was compared morphologically with several species collected from the Neotropics (Chaverri et al. 2008), China (Qiu et al. 2009, Qiu & Guan 2010), and Thailand (Luangsa-ard et al. 2007, 2008, 2010, Mongkolsamrit et al. 2009). The Vietnamese specimens are mostly similar to *Aschersonia andropogonis* (teleomorph: *M. ochracea*) in having pulvinate stroma, yellow, yellowish orange to orange spore masses, fusiform conidia (8–14 × 1.5–2 µm), which is commonly found in the Neotropics. However, *M. pumatensis* differs from *A. andropogonis* in the color of the stroma. *Moelleriella pumatensis* has a golden yellow stroma while that of *A. andropogonis* is white to pale yellow. The DNA sequence analysis of translation elongation factor 1-α (*tef1*) also supports *M. pumatensis* as a new species.

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